

=&gt; d que stat l10

L1 7 SEA FILE=REGISTRY ABB=ON (IRON OR CALCIUM OR ALUMINUM OR NICKEL OR ZINC OR COBALT OR COPPER)/CN

L2 111827 SEA FILE=HCAPLUS ABB=ON (?PEPTID? OR ?PROTEIN?) (4A) (?PURIF? OR ?WASH? OR ?EXTRACT? OR ?ELUT?)

L3 3656 SEA FILE=HCAPLUS ABB=ON L2 AND (?CHELAT? OR ?RESIN?)

L4 1288 SEA FILE=HCAPLUS ABB=ON L3 AND (L1 OR ?IRON? OR FE OR ?CALCIUM? OR CA OR ?ALUMINUM? OR AL OR ?NICKEL? OR NI OR ?ZINC? OR ZN OR ?COBALT? OR CO OR ?COPPER? OR CU)

L5 229 SEA FILE=HCAPLUS ABB=ON L4 AND (?FUSE? OR ?FUSION?)

L6 177 SEA FILE=HCAPLUS ABB=ON L5 AND (?CHROMATOG? OR ?BUFFER? OR ?COLUMN?)

L7 4 SEA FILE=HCAPLUS ABB=ON L6 AND (?HARD? OR ?INTERMED?)

L8 6 SEA FILE=HCAPLUS ABB=ON L6 AND KIT?

L9 9 SEA FILE=HCAPLUS ABB=ON L7 OR L8

L10 9 SEA FILE=HCAPLUS ABB=ON L7 OR L8 OR L9

=&gt; d ibib abs l10 1-9

L10 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:34869 HCAPLUS

TITLE: Methods and compositions for enhanced **protein** expression and **purification**

INVENTOR(S): Butt, Tauseef R.; Malakhov, Michael P.; Malakhova, Oxana A.

PATENT ASSIGNEE(S): Lifesensors, Inc., USA

SOURCE: PCT Int. Appl., 107 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005003313	A2	20050113	WO 2004-US20778	20040628
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 2003-482817P P 20030626

AB Methods for enhancing expression levels and secretion of heterologous **fusion** proteins in a host cell are disclosed. The method comprises: mol. cloning of COOH-terminal of a Ubl mol. selected from the group consisting of SUMO, UB, RUB, HIB, APG8, APG12, URM1, Ubi-L, FAT10 and ISG15 to a nucleic acid sequence encoding the protein of interest. The invention further relates to the compns. and **purification** of the **protein**.

L10 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:905784 HCAPLUS

DOCUMENT NUMBER: 141:376819

TITLE: Affinity purification system using troponin molecules

as affinity ligands  
 INVENTOR(S): Moeckli, Randolph A.; Chadwick, Christopher C.  
 PATENT ASSIGNEE(S): Aristex, USA  
 SOURCE: PCT Int. Appl., 68 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004092214	A2	20041028	WO 2004-US10887	20040407
WO 2004092214	A3	20041118		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 2003-462483P P 20030410

AB This invention pertains to the use of a novel set of tags for the immobilization and/or **purification** of **proteins** or other biol. or organic mols. The invention provides troponin C, troponin C binding peptide, and troponin I, or active fragments or analogs thereof as convenient tags and affinity ligands for immobilizing, attaching, or **purifying proteins** or other mols. Methods for producing troponin-tagged mols., such as recombinant **fusion** proteins, are described. Methods for preparing a troponin affinity matrix that is capable of specifically binding its cognate ligand in the presence of **calcium**, and methods for using such a matrix to purify troponin-tagged mols. are also described.

L10 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:759732 HCAPLUS

DOCUMENT NUMBER: 141:273989

TITLE: **Purification of fusion proteins** using immobilized bi-metal affinity **chromatography**

INVENTOR(S): Tchaga, Grigoriy S.; Jokhadze, George G.

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 20 pp., Cont.-in-part of U.S. Pat. Appl. 2002 164,718.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004180415	A1	20040916	US 2004-762588	20040121
US 2002164718	A1	20021107	US 2001-858332	20010515
PRIORITY APPLN. INFO.:			US 2001-858332	A2 20010515
			US 2003-441804P	P 20030121

US 1998-101867P P 19980925  
US 1999-404017 B2 19990923

AB The present invention relates to IMAC (Immobilized Metal Affinity Chromatog.). The present invention provides methods of purifying proteins that include a metal ion affinity peptide. The methods generally involve contacting a fusion protein that includes a metal ion affinity peptide with at least two different metal ion chelating resins. In certain representative embodiments, the methods include contacting a fusion protein with a first metal ion chelate resin having a first immobilized metal ion; eluting any bound protein from the first metal ion chelate resin, to produce an eluate; contacting the eluate with a second metal ion chelate resin having a second immobilized metal ion; and eluting any bound protein from the second metal ion chelate resin. Also provided are kits for use in practicing the subject methods. An illustrative purification protocol for Bi-MAC (Bi-Metal Affinity Chromatog.) is shown. The subject methods find use in a variety of protein purification applications.

L10 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:790100 HCAPLUS

DOCUMENT NUMBER: 140:176405

TITLE: Molecular cloning, overexpression in Escherichia coli, and purification of 6+ his-tagged C-terminal domain of Clostridium difficile toxins A and B

AUTHOR(S): Letourneur, Odile; Ottone, Sophie; Delauzun, Vincent; Bastide, Marie-Claire; Foussadier, Agnes

CORPORATE SOURCE: BioMerieux, Chemin de l'Orme, Marcy l'Etoile, 69280, Fr.

SOURCE: Protein Expression and Purification (2003), 31(2), 276-285

CODEN: PEXPEJ; ISSN: 1046-5928

PUBLISHER: Elsevier Science

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Genomic DNA from ribotype-01 and -17 Clostridium difficile strains was used for amplification of the sequences encoding the carboxy-terminal domain of toxins A (TcdA) and B (TcdB). The deduced C-terminal TcdB ribotype-01 and -17 domains share 99.5% amino acid sequence identity while TcdA ribotype-17 comprises a 607 amino acid deletion compared to TcdA-01. When compared to previously sequenced C. difficile toxins, 99.3% amino acid identity was found between TcdA-01 and TcdA from strain VPI10643 and 98.8% identity between TcdA-17 and TcdA from strain F-1470. The obtained sequences were fused in 3' to a sequence encoding a hexahistidine tag and cloned into an Escherichia coli expression vector. The recombinant proteins were expressed in E. coli and purified using single-step metal-chelate chromatog. The recombinant carboxy-terminal domain of TcdA-01 was purified from the soluble E. coli lysate fraction whereas TcdA-17 and TcdB-17 carboxy-terminal domains were purified from inclusion bodies. At least 40 mg of each protein was purified per L of bacterial culture. The recombinant toxin domains were detected specifically by Western blot and ELISA with antibodies against native C. difficile toxins. This study demonstrated that the carboxy-terminal domains of TcdA and TcdB can be produced using an E. coli expression system and easily purified. These recombinant, stable, and non-toxic proteins provide a convenient source for use in the diagnosis of C. difficile infections, instead of native toxins, as controls and calibrators in immunoassay kits and to obtain

specific monoclonal antibodies.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:78239 HCAPLUS

DOCUMENT NUMBER: 136:133614

TITLE: purification and application of IgY (IgY Fc) from egg yolk of immunized duck

INVENTOR(S): Chiu, Yi-neng

PATENT ASSIGNEE(S): Kute Bioscience and Technology Y. K., Taiwan

SOURCE: Jpn. Kokai Tokkyo Koho, 15 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002030100	A2	20020129	JP 2000-200544	20000703
JP 3610551	B2	20050112		

PRIORITY APPLN. INFO.: JP 2000-200544 20000703

AB A method is provided to purify IgY from egg yolk of duck immunized with antigens. In order to avoid the immunogenicity and unnecessary complement activation, it is an advanced development to delete or limit the responsible Fc fragment of the antibody. The antigen-specific IgY (IgY Fc) purified from egg yolk by using immuno-affinity method under conditions such as PH 4-7 and lower than 50 mM of ion concentration provides the possible application in therapy and diagnosis of diseases.

L10 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:508761 HCAPLUS

DOCUMENT NUMBER: 131:254057

TITLE: Selective adsorption of poly-His tagged glutaryl acylase on tailor-made metal **chelate** supports

AUTHOR(S): Armisen, Pilar; Mateo, Cesar; Cortes, Estrella; Barredo, Jose L.; Salto, Francisco; Diez, Bruno; Rodes, Lorenzo; Garcia, Jose L.; Fernandez-Lafuente, Roberto; Guisan, Jose M.

CORPORATE SOURCE: Hispanagar S.A., Burgos, Spain

SOURCE: Journal of Chromatography, A (1999), 848(1 + 2), 61-70  
CODEN: JCRAEY; ISSN: 0021-9673

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A poly-His tag was **fused** in the glutaryl acylase (GA) from *Acinetobacter* sp. strain YS114 cloned in *E. coli* yielding a fully active enzyme. Biochem. analyses showed that the tag did not alter the maturation of the chimeric GA (poly-His GA) that undergoes a complex post-translational processing from an inactive monomeric precursor to the active heterodimeric enzyme. This enzyme has been used as a model to develop a novel and very simple procedure for one-step **purification** of poly-His **proteins** via immobilized metal-ion affinity chromatog. on tailor-made supports. It was intended to improve the selectivity of adsorption of the target protein on tailor-made **chelate** supports instead of performing a selective desorption. The rate and extent of the adsorption of **proteins** from a crude

extract from E. coli and of pure poly-His tagged GA on different metal chelate supports was studied. Up to 90% of proteins from E. coli were adsorbed on com. chelate supports having a high d. of ligands attached to the support through long spacer arms, while this adsorption becomes almost negligible when using low ligand densities, short spacer arms and Zn<sup>2+</sup> or Co<sup>2+</sup> as cations. On the contrary, poly-His GA adsorbs strongly enough on all supports. A strong affinity interaction between the poly-His tail and a single chelate moiety seems to be the responsible for the adsorption of poly-His GA. By contrast, multipoint weak interactions involving a number of chelate moieties seem to be mainly responsible for adsorption of natural proteins. By using tailor-made affinity supports, a very simple procedure for one-step purification of GA with minimal adsorption of host proteins could be performed. Up to 20 mg of GA were adsorbed on each ml of chelate support while most of accompanying proteins were hardly adsorbed on such supports. Following few washing steps, the target enzyme was finally recovered (80% yield) by elution with 50 mM imidazole with a very high increment of specific activity (up to a 120 purification factor).

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1998:151235 HCAPLUS

DOCUMENT NUMBER: 128:177564

TITLE: Recombinant myeloperoxidase (MPO) fragment and methods for assaying MPO-specific anti-neutrophil cytoplasmic autoantibody (MPO-ANCA) reaction site and determining MPO-ANCA by using the fragment

INVENTOR(S): Suzuki, Kazuo; Tanokura, Masaru

PATENT ASSIGNEE(S): Teikoku Hormone Mfg. Co., Ltd., Japan; Suzuki, Kazuo; Tanokura, Masaru

SOURCE: PCT Int. Appl., 46 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9807848	A1	19980226	WO 1997-JP2910	19970821
W: AU, CA, CN, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9738677	A1	19980306	AU 1997-38677	19970821
PRIORITY APPLN. INFO.:			JP 1996-219855	A 19960821
			WO 1997-JP2910	W 19970821

AB Disclosed are a recombinant MPO fragment reacting with myeloperoxidase-specific anti-neutrophil cytoplasmic antibody (MPO-ANCA); a process for producing the fragment by expression of the encoding cDNA in Escherichia coli; a method for assaying the MPO-ANCA reaction site and its amount in a specimen by using the fragment; and reagent kits for the assay which consists of a panel set of the fragment and labeled anti-human IgG. PCR amplification to obtain the cDNA encoding 16 human MPO fragments, preparation of MPO fragments fused with 6 Histidines in transgenic Escherichia coli, and purification of the fusion protein by using Ni-NTA (Nickel-nitrilotriacetate) resin column were shown. The MPO fragments are useful in diagnosis of autoimmune diseases.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1997:104290 HCAPLUS

DOCUMENT NUMBER: 126:195152

TITLE: Naloxone-Sensitive, haloperidol-sensitive,  
[3H](+)SKF-10047-binding **protein** partially  
**purified** from rat liver and rat brain  
membranes: An opioid/Sigma receptor?

AUTHOR(S): Tsao, Li-I.; Su, Tsung-Ping

CORPORATE SOURCE: Molecular Neuropsychiatry Section, Division of  
Intramural Research, National Institute on Drug  
Abuse/NIH, Baltimore, MD, USA

SOURCE: Synapse (New York) (1997), 25(2), 117-124

CODEN: SYNAET; ISSN: 0887-4476

PUBLISHER: Wiley-Liss

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A naloxone-sensitive, haloperidol-sensitive, [3H](+)SKF-10047-binding **protein** was partially **purified** from rat liver and rat brain membranes in an affinity **chromatog.** originally designed to purify sigma receptors. Detergent-solubilized exts. from membranes were adsorbed to Sephadex G-25 **resin** containing an affinity ligand for sigma receptors: N-(2-[3,4-dichlorophenyl]ethyl)-N-(6-aminohexyl)-(2-[1-pyrrolidinyl])ethylamine (DAPE). After eluting the **resin** with haloperidol, a protein that bound [3](+)SKF-10047 was detected in the eluates. However, the protein was not the sigma receptor. [3H](+)SKF-10047 binding to the protein was inhibited by the following compds. in the order of decreasing potency: (+)pentazocine > (-)pentazocine > (+)cyclazocine > (-)morphine > (-)naloxone > haloperidol > (+)SKF-10047 > DADLE > (-)SKF-10047. Further, the prototypic sigma receptor ligands, such as 1,3-di-o-tolylguanidine (DTG), (+)3-PPP, and progesterone, bound poorly to the protein. Tryptic digestion and heat treatment of the affinity-**purified protein** abolished radioligand binding. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) of the partially-**purified protein** from the liver revealed a major **diffuse** band with a mol. mass of 31 kDa, a polypeptide of 65 kDa, and another polypeptide of > 97 kDa. This study demonstrates the existence of a novel protein in the rat liver and rat brain which binds opioids, benzomorphans, and haloperidol with nanomolar affinity. The protein resembles the opioid/sigma receptor originally proposed by Martin et al. [(1976): J. Pharmacol. Exp. Ther., 197:517-532.]. A high degree of **purification** of this **protein** has been achieved in the present study.

L10 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1996:706909 HCAPLUS

DOCUMENT NUMBER: 126:15280

TITLE: A multifunctional vector system for heterologous  
expression of proteins in Escherichia coli. Expression  
of native and hexahistidyl **fusion**  
**proteins**, rapid **purification** of the  
**fusion proteins**, and removal of  
**fusion** peptide by Kex2 protease

AUTHOR(S): Ghosh, Smita; Lowenstein, John M.

CORPORATE SOURCE: Biochemistry Department, Brandeis University, Waltham,  
MA, 02254, USA

SOURCE: Gene (1996), 176(1/2), 249-255

CODEN: GENED6; ISSN: 0378-1119

PUBLISHER: Elsevier

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Vectors have been constructed for the general purpose of expressing foreign proteins in *E. coli*. These vectors allow the production in high yield of either native proteins or of **fusion** proteins which contain, at their amino terminus, the peptide Met Gly His6 Ser Gly Leu Phe Lys Arg/, where Leu Phe Lys Arg/ is the recognition site for Kex2 protease which cleaves at the site indicated by /. The His6 sequence is used as a ligand for the one-step affinity **purification** of the expressed **proteins on columns** containing **Ni** or **Zn** ions **chelated** to iminodiacetic acid-agarose. After affinity **chromatog.**, the **purification peptide** is cleaved off with Kex2 protease from *Saccharomyces cerevisiae*. The vectors also allow site-directed mutagenesis and sequencing of the cloned gene to be expressed without any **intermediate** subcloning. For practical examples of over-expression, affinity purification, and removal of the **purification peptide**, the authors chose a high-mol.-weight protein, phospholipase C $\gamma$ 1 (PLC $\gamma$ 1, Mr 148 000) and a low-mol.-weight protein, Hit-1 (Mr 16 000). Both were obtained pure and in high yield. PLC $\gamma$ 1 was fully active; the function of Hit-1 is not known. A set of companion vectors for **co-expression** of addnl. proteins has also been developed. These allow expression of proteins which enhance the production of activity of the protein of primary interest and of proteins which exhibit trans-interactions.

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L1          7 SEA FILE=REGISTRY ABB=ON (IRON OR CALCIUM OR ALUMINUM OR
          NICKEL OR ZINC OR COBALT OR COPPER)/CN
L2          111827 SEA FILE=HCAPLUS ABB=ON (?PEPTID? OR ?PROTEIN?) (4A) (?PURIF?
          OR ?WASH? OR ?EXTRACT? OR ?ELUT?)
L3          3656 SEA FILE=HCAPLUS ABB=ON L2 AND (?CHELAT? OR ?RESIN?)
L4          1288 SEA FILE=HCAPLUS ABB=ON L3 AND (L1 OR ?IRON? OR FE OR
          ?CALCIUM? OR CA OR ?ALUMINUM? OR AL OR ?NICKEL? OR NI OR
          ?ZINC? OR ZN OR ?COBALT? OR CO OR ?COPPER? OR CU)
L5          229 SEA FILE=HCAPLUS ABB=ON L4 AND (?FUSE? OR ?FUSION?)
L6          177 SEA FILE=HCAPLUS ABB=ON L5 AND (?CHROMATOG? OR ?BUFFER? OR
          ?COLUMN?)
L7          4 SEA FILE=HCAPLUS ABB=ON L6 AND (?HARD? OR ?INTERMED?)
L8          6 SEA FILE=HCAPLUS ABB=ON L6 AND KIT?
L9          9 SEA FILE=HCAPLUS ABB=ON L7 OR L8
L10         9 SEA FILE=HCAPLUS ABB=ON L7 OR L8 OR L9
L11         15 SEA L10
L12         13 DUP REMOV L11 (2 DUPLICATES REMOVED)

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=> d ibib abs l12 1-13

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L12 ANSWER 1 OF 13 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
    on STN
ACCESSION NUMBER: 2004282234 EMBASE
TITLE: Detection of IgG antibody to Toxoplasma gondii with rSAG1
    for immunodiagnosis of toxoplasmosis.
AUTHOR: Si J.; Tang J.-M.; Xu M.; Yang X.-H.; Yin X.-R.; Cao G.-Q.;
    Liang Y.-S.; Dai J.-R.; Zhu Y.-C.
CORPORATE SOURCE: J. Si, Jiangsu Inst. of Parasitic Diseases, Wuxi 214064,
    China. sijin2001@sohu.com
SOURCE: Chinese Journal of Microbiology and Immunology, (2004) 24/3
    (245-248).
    Refs: 10
    ISSN: 0254-5101 CODEN: ZWMZDP
COUNTRY: China
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
    005 General Pathology and Pathological Anatomy
    026 Immunology, Serology and Transplantation
LANGUAGE: Chinese
SUMMARY LANGUAGE: English; Chinese
AB Objective: To establish a rSAG1-ELISA method with purified rSAG1
fusion protein for immunodiagnosis of toxoplasmosis.
Methods: rSAG1 fusion protein was expressed and
purified by Ni(2+) chelating Hi-Trap HP
column. ELISA plate was coated with different concentrations of
rSAG1, reacted with pooled positive and negative sera, Goat anti-human IgG
conjugated to horseradish peroxidase was used as a second antibody. The
appropriate detecting condition of rSAG1-ELISA assay was determined by
orthogonal experiment, the reproducibility, sensitivity and specificity of
the assay were tested. Fifty-two IgG-positive and 40 IgG-negative human
sera detected with imported IgG-ELISA kit were tested with
rSAG1-ELISA. Results: The purity of rSAG1 was above 90%; the appropriate
detecting condition of rSAG1-ELISA was that the concentration of coated
rSAG1 must be 5µg/ml, the human serum should be 1: 100 diluted, and
goat anti-human IgG conjugated to horseradish peroxidase was in 1: 2000
dilution; the CV value for IgG-positive and IgG-negative human sera were
10.9% and 10.7% respectively; the inhibition rate of the assay was 62.6%;
the total correspondence rate of rSAG1-ELISA was 87% with imported
IgG-ELISA kit. The positive correspondence rate and negative

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correspondence rate of rSAG1-ELISA was 81% (42/52) and 95% (38/40) respectively as compared with imported IgG-ELISA **kit** of toxoplasmosis. Conclusion: The rSAG1-ELISA has high sensitivity and specificity and good correspondence rate with imported IgG-ELISA **kit** of toxoplasmosis. rSAG1-ELISA has potential value for immunodiagnosis of toxoplasmosis.

L12 ANSWER 2 OF 13 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2002148415 EMBASE  
TITLE: A study of recombinant protective H. pylori antigens.  
AUTHOR: Jiang Z.; Tao X.-H.; Huang A.-L.; Wang P.-L.  
CORPORATE SOURCE: Dr. Z. Jiang, Department of Gastroenterology, First  
Affiliated Hospital, Chongqing Univ. of Medical Sciences,  
Chongqing 400016, China. jzh053@mail.china.com  
SOURCE: World Journal of Gastroenterology, (2002) 8/2 (308-311).  
Refs: 32  
ISSN: 1007-9327 CODEN: WJGAF2  
COUNTRY: China  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
026 Immunology, Serology and Transplantation  
030 Pharmacology  
025 Hematology  
037 Drug Literature Index  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Aim: To construct a recombinant vector which can express M(r) 26 000 outer membrane protein (OMP) from Helicobacter pylori (Hp), and to obtain the vaccine protecting against Hp infection and a diagnostic reagent **kit** quickly detecting Hp infection. Methods: The gene encoding the structural M(r) 26 000 outer membrane protein of Hp was amplified from Hp chromosomal DNA by PCR, and inserted in the prokaryotic expression vector pET32a (+), which was transformed into the Top10 E. coli strain. Recombinant vector was selected, identified and transformed into BL-21 (DE3) E. coli strain. The recombinant **fusion** proteins were expressed. The antigenicity of recombinant protein was studied by ELISA or immunoblotting and immunized Balb/c mice. Results: The gene of M(r) 26 000 OMP was amplified to be 594 base pairs, 1.1% of the cloned genes was mutated and 1.51% of amino acid residues was changed, but there was homogeneity between them. The recombinant **fusion** protein encoded objective polypeptides of 198 amino acid residues, corresponding to calculated molecular masses of M(r) 26 000. The level of soluble expression products was about 38.96% of the total cell **protein**. After **purification** by Ni-NTA agarose **resin** **columniation**, the purity of objective protein became about 90%. The ELISA results showed that recombinant **fusion** protein could be recognized by patient serum infected with Hp and rabbit serum immunized with the recombinant protein. Furthermore, Balb/ c mice immunized with the recombinant protein were protected against H. pylori infection. Conclusion: Mr 26 000 OMP may be a candidate vaccine preventing Hp infection.

L12 ANSWER 3 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on  
STN

ACCESSION NUMBER: 2002:502155 BIOSIS  
DOCUMENT NUMBER: PREV200200502155  
TITLE: A comparative study of the human T-cell leukemia virus type 2 integrase expressed in and purified from Escherichia coli and Pichia pastoris.

AUTHOR(S): Piefer, Andrew J.; Jonsson, Colleen B. [Reprint author]  
 CORPORATE SOURCE: Department of Chemistry and Biochemistry, New Mexico State  
 University, Las Cruces, NM, 88003, USA  
 cjonsson@nmsu.edu

SOURCE: Protein Expression and Purification, (July, 2002) Vol. 25,  
 No. 2, pp. 291-299. print.  
 CODEN: PEXPEJ. ISSN: 1046-5928.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 25 Sep 2002

Last Updated on STN: 25 Sep 2002

AB The human T-cell leukemia virus type-2 (HTLV-2) integrase (IN) catalyzes the insertion of the viral genome into the host chromosome. HTLV-2 IN was expressed as an N-terminal hexa-histidine tagged protein in the methylotrophic yeast *Pichia pastoris* and as a C-terminal hexa-histidine fusion in *Escherichia coli*. Maximal IN expression was observed at 48 h post-induction for the yeast system and 2 h post-induction for *E. coli*. Effective purification strategies were developed using non-ionic and zwitterionic detergents for initial **protein extraction**, followed by a one-step **nickel-chelating chromatography** purification. IN from both sources was routinely greater than 90% pure with yields exceeding 1.5 mg of purified IN per liter of culture for *P. pastoris*. The relative pI was defined for both INs, pH 5.0-5.4, by 2D-gel electrophoresis. Specific activities for IN purified from *E. coli* and *P. pastoris* were calculated from in vitro 3' processing assays and were comparable. In vitro IN assays were also performed to optimize reaction **buffer** pH and metal concentrations for both 3' processing and strand transfer assays. Strand transfer was optimal from pH 6.2-6.8, more than 1.5 pH units below the optimal 3' processing pH of 8.3. IN from both sources showed no enhancement in activity with MnCl<sub>2</sub> concentrations greater than 5 mM. The specific activity of *P. pastoris* purified IN was 0.35 product (pmol)/h/mug IN, and *E. coli* produced IN was 0.48 product (pmol)/h/mug IN.

L12 ANSWER 4 OF 13

MEDLINE on STN

ACCESSION NUMBER: 2002045382 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11755059

TITLE: Expression of ecdysteroid receptor and ultraspiracle from **Chironomus tentans** (Insecta, Diptera) in *E. coli* and purification in a functional state.

AUTHOR: Grebe Marco; Spindler-Barth Margarethe

CORPORATE SOURCE: Abteilung fur Allgemeine Zoologie und Endokrinologie,  
 Universitat Ulm, Albert-Einstein-Allee 11, 89081 Ulm,  
 Germany.

SOURCE: Insect biochemistry and molecular biology, (2002 Feb) 32  
 (2) 167-74.

Journal code: 9207282. ISSN: 0965-1748.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200203

ENTRY DATE: Entered STN: 20020124

Last Updated on STN: 20020313

Entered Medline: 20020312

AB Full length clones of ecdysteroid receptor (EcR) and Ultraspiracle (USP) from **Chironomus tentans** were expressed as GST **fusion proteins** in *E. coli* and **purified** by affinity **chromatography**. The absence of detergents during the purification procedure is essential for retaining receptor function, especially ligand

binding. Presence of USP is mandatory for ligand binding to EcR, but no other cofactors or posttranslational modifications seem to be important, since **Scatchard** plots revealed the same characteristics (two high affinity binding sites for Ponasterone A with  $K(D1)=0.24\pm 0.1\text{ nM}$  and  $K(D2)=3.9\pm 1.3\text{ nM}$ ) as found in 0.4 M NaCl extracts of **Chironomus** cells. Gel mobility shift assays showed binding of the heterodimer to PAL and DR5 even after removal of the GST-tag, whereas EcR binding to PAL1 is GST-dependent. USP binds preferentially to DR5. Addition of unprogrammed reticulocyte lysate improves ligand binding only slightly. Removal of GST has no effect on (3)H-ponasterone A binding, but alters DNA binding characteristics. Calculation of specific binding ( $5.3\pm 3.0\text{ nmol/mg GST EcR}$ ) revealed that  $47\pm 26\%$  of **purified receptor protein** was able to bind ligand. The addition of purified EcR to cell extracts of hormone resistant subclones of the epithelial cell line from C. tentans, which have lost their ability to bind ligand, restores specific binding of (3)H-ponasterone A.

L12 ANSWER 5 OF 13 MEDLINE on STN  
 ACCESSION NUMBER: 2002019721 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 11437613  
 TITLE: SwellGel: an affinity **chromatography** technology for high-capacity and high-throughput **purification** of recombinant-tagged **proteins**.  
 AUTHOR: Draveling C; Ren L; Haney P; Zeisse D; Qoronfleh M W  
 CORPORATE SOURCE: Perbio Sciences, 2202 N. Bartlett Avenue, Milwaukee, Wisconsin, 53202-1009, USA.  
 SOURCE: Protein expression and purification, (2001 Jul) 22 (2) 359-66.  
 Journal code: 9101496. ISSN: 1046-5928.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200112  
 ENTRY DATE: Entered STN: 20020121  
 Last Updated on STN: 20020121  
 Entered Medline: 20011207  
 AB The revolution in genomics and proteomics is having a profound impact on drug discovery. Today's protein scientist demands a faster, easier, more reliable way to **purify proteins**. A high capacity, high-throughput new technology has been developed in Perbio Sciences for affinity **protein purification**. This technology utilizes selected **chromatography** media that are dehydrated to form uniform aggregates. The SwellGel aggregates will instantly rehydrate upon addition of the **protein** sample, allowing **purification** and direct performance of multiple assays in a variety of formats. SwellGel technology has greater stability and is easier to handle than standard wet **chromatography resins**. The microplate format of this technology provides high-capacity, high-throughput features, recovering milligram quantities of protein suitable for high-throughput screening or biophysical/structural studies. Data will be presented applying SwellGel technology to recombinant 6x His-tagged protein and glutathione-S-transferase (GST) **fusion protein purification**.  
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L12 ANSWER 6 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN  
 ACCESSION NUMBER: 2001:378784 BIOSIS  
 DOCUMENT NUMBER: PREV200100378784

TITLE: Affinity of the monoclonal antibody M1 directed against the FLAG peptide.  
AUTHOR(S): Einhauer, A.; Jungbauer, A. [Reprint author]  
CORPORATE SOURCE: Institute of Applied Microbiology, University of Agricultural Sciences, Muthgasse 18, A-1190, Vienna, Austria  
SOURCE: jungbaue@hp01.boku.ac.at  
Journal of Chromatography A, (29 June, 2001) Vol. 921, No. 1, pp. 25-30. print.  
CODEN: JOCRAM. ISSN: 0021-9673.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 8 Aug 2001  
Last Updated on STN: 19 Feb 2002

AB The FLAG (Sigma, St. Louis, MO, USA) peptide is a frequently used hydrophilic and immunogenic **fusion** tag which was specifically designed to facilitate rapid purification by immunoaffinity **chromatography**. The monoclonal antibody M1 recognizes the free N-terminus of the peptide tag in a **calcium** dependent manner. Dissociation of the complex can be performed by the addition of **chelating** agents such as EDTA. This effect can be exploited for immunoaffinity **purification** of FLAG-tagged **fusion proteins**. Kinetic information obtained from monitoring interactions in real-time measurement (Biacore 2000) using surface plasmon resonance as detection principle did not show any difference for association and dissociation rate constants in the presence ( $k_a = 3.03 \text{ cntdot} 10^3 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_d = 1.25 \text{ cntdot} 10^{-3} \text{ s}^{-1}$ ) and in the absence of  $\text{Ca}^{2+}$  ( $k_a = 3.59 \text{ cntdot} 10^3 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_d = 1.16 \text{ cntdot} 10^{-3} \text{ s}^{-1}$ ). These findings corroborate the reports from Mol. Immunol. 33 (1996) 601-608 describing similar binding analyzed by enzyme-linked immunosorbent assay experiments. These investigations are in contrast to the observations in immunoaffinity **chromatography** with immobilized anti-FLAG antibody M1.

L12 ANSWER 7 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:233629 BIOSIS  
DOCUMENT NUMBER: PREV200000233629  
TITLE: Expression in Escherichia coli and simple **purification** of human Fhit **protein**.  
AUTHOR(S): Pawelczyk, Tadeusz [Reprint author]; Kowara, Renata; Golebiowski, Filip; Matecki, Andrzej  
CORPORATE SOURCE: Department of Molecular Medicine, Medical University of Gdansk, 80-211, Gdansk, Poland  
SOURCE: Protein Expression and Purification, (April, 2000) Vol. 18, No. 3, pp. 320-326. print.  
CODEN: PEXPEJ. ISSN: 1046-5928.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
OTHER SOURCE: Genbank-U46922  
ENTRY DATE: Entered STN: 7 Jun 2000  
Last Updated on STN: 5 Jan 2002

AB The fragile histidine triad (Fhit) protein is a homodimeric protein with diadenosine 5',5'''-P1,P3-triphosphate (Ap3A) asymmetrical hydrolase activity. We have cloned the human cDNA Fhit in the pPROEX-1 vector and expressed with high yield in Escherichia coli with the sequence Met-Gly-His6-Asp-Tyr-Asp-Ile-Pro-Thr-Thr followed by a rTEV protease cleavage site, denoted as "H6TV," **fused** to the N-terminus of Fhit. Expression of H6TV-Fhit in BL21(DE3) cells for 3 h at 37degreeC produced 30 mg of H6TV-Fhit from 1 L of cell culture (apprx4 g of cells). The H6TV-Fhit **protein** was **purified** to homogeneity in a

single step, with a yield of 80%, using **nickel-nitrilotriacetate resin** and imidazole **buffer** as eluting agent. Incubation of H6TV-Fhit with rTEV protease at 4degreeC for 24 h resulted in complete cleavage of the H6TV peptide. There were no unspecific cleavage products. The **purified Fhit protein** could be stored for 3 weeks at 4degreeC without loss of activity. The pure protein was stable at -20degreeC for at least 18 months when stored in **buffer** containing 25% glycerol. Purified Fhit was highly active, with a Km value for Ap3A of 0.9 muM and a kcat(monomer) value of 7.2 +- 1.6 s<sup>-1</sup> (n = 5). The catalytic properties of unconjugated Fhit protein and the H6TV-Fhit **fusion** protein were essentially identical. This indicates that the 24-amino-acid peptide containing the six histidines **fused** to the N-terminus of Fhit does not interfere in forming the active homodimers or in the binding of Ap3A.

L12 ANSWER 8 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:233628 BIOSIS  
DOCUMENT NUMBER: PREV200000233628  
TITLE: Expression of human cardiac-specific homeobox protein in Escherichia coli.  
AUTHOR(S): Zhao, Jing-Hui; Xu, Zhao; Hua, Zi-Chun [Reprint author]  
CORPORATE SOURCE: State Key Laboratory of Pharmaceutical Biotechnology, Department of Biochemistry, School of Life Sciences, Nanjing University, Nanjing, 210093, China  
SOURCE: ~~Protein Expression and Purification, (April, 2000) Vol. 18, No. 3, pp. 316-319. print.~~  
CODEN: PEXPEJ. ISSN: 1046-5928.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 7 Jun 2000  
Last Updated on STN: 5 Jan 2002

AB Human cardiac-specific homeobox protein cDNA (hCsx) was cloned into expression plasmid pET32a and **fused** with Escherichia coli thioredoxin (Trx). The Trx-Csx **fusion** protein was under the control of bacteriophage T7 promoter. When expressed in E. coli BL21(DE3), about half of the recombinant Trx-Csx products existed in the form of insoluble inclusion bodies. When coexpressed with human protein disulfide isomerase, more than 90% of Trx-Csx products accumulated in the soluble form in the cell lysate. The recombinant Csx **fusion protein was purified** by one-step metal-chelating affinity **chromatography**.

L12 ANSWER 9 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:220905 BIOSIS  
DOCUMENT NUMBER: PREV200000220905  
TITLE: **Purification of His-tagged proteins by immobilized chelate affinity chromatography**: The benefits from the use of organic solvent.  
AUTHOR(S): Franken, Kees L. M. C. [Reprint author]; Hiemstra, Hoebert S. [Reprint author]; van Meijgaarden, Krista E. [Reprint author]; Subronto, Yanri [Reprint author]; den Hartigh, J.; Ottenhoff, Tom H. M. [Reprint author]; Drijfhout, Jan W. [Reprint author]  
CORPORATE SOURCE: Department of Immunohematology and Blood Bank, Leiden University Medical Center, 2300 RC, Leiden, Netherlands  
SOURCE: ~~Protein Expression and Purification, (Feb., 2000) Vol. 18, No. 1, pp. 95-99. print.~~

CODEN: PEXPEJ. ISSN: 1046-5928.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 31 May 2000  
Last Updated on STN: 5 Jan 2002

AB Recombinant **proteins** overexpressed in and **purified** from *Escherichia coli* contain impurities that are toxic in biological assays. The application of affinity purification procedures is often not sufficient to remove these toxic components. We here describe a simple and fast, one-step protocol to remove these impurities highly efficiently. Four recombinant proteins were overexpressed in *E. coli* as His-tagged **fusion proteins** and **purified** by immobilized metal **chelate** affinity **chromatography** on Ni-NTA beads. Depending on the protein, the composition of the lysis **buffer**, and the washing protocol, various impurities appeared to be present in the **purified protein** preparations. Here we show how the use of 60% isopropanol during washing steps removed most of these contaminants from the end products. In addition to the removal of proteins that aspecifically adhere to the beads or to the tagged protein, this procedure was particularly useful in removing endotoxins. Moreover, we show that detergents such as NP-40, that are necessarily employed during lysis, are also efficiently removed. Finally, we show that proteins are able to refold correctly after isopropanol treatment. Thus, the resulting end products contain significantly less contaminating *E. coli* proteins, endotoxins, and detergents.

L12 ANSWER 10 OF 13 MEDLINE on STN  
ACCESSION NUMBER: 1999356759 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10427752  
TITLE: Selective adsorption of poly-His tagged glutaryl acylase on tailor-made metal **chelate** supports.  
AUTHOR: Armisen P; Mateo C; Cortes E; Barredo J L; Salto F; Diez B; Rodes L; Garcia J L; Fernandez-Lafuente R; Guisan J M  
CORPORATE SOURCE: Hispanagar S.A., Burgos, Spain.  
SOURCE: Journal of chromatography. A, (1999 Jul 2) 848 (1-2) 61-70.  
Journal code: 9318488.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199908  
ENTRY DATE: Entered STN: 19990913  
Last Updated on STN: 20020420  
Entered Medline: 19990830

AB A poly-His tag was **fused** in the glutaryl acylase (GA) from *Acinetobacter* sp. strain YS114 cloned in *E. coli* yielding a fully active enzyme. Biochemical analyses showed that the tag did not alter the maturation of the chimeric GA (poly-His GA) that undergoes a complex post-translational processing from an inactive monomeric precursor to the active heterodimeric enzyme. This enzyme has been used as a model to develop a novel and very simple procedure for one-step **purification** of poly-His **proteins** via immobilized metal-ion affinity **chromatography** on tailor-made supports. It was intended to improve the selectivity of adsorption of the target protein on tailor-made **chelate** supports instead of performing a selective desorption. The rate and extent of the adsorption of **proteins** from a crude **extract** from *E. coli* and of pure poly-His tagged GA on different metal **chelate** supports was studied. Up to 90% of proteins from *E. coli* were adsorbed on commercial **chelate** supports having a high density of ligands attached to the

support through long spacer arms, while this adsorption becomes almost negligible when using low ligand densities, short spacer arms and Zn<sup>2+</sup> or Co<sup>2+</sup> as cations. On the contrary, poly-His GA adsorbs strongly enough on all supports. A strong affinity interaction between the poly-His tail and a single **chelate** moiety seems to be the responsible for the adsorption of poly-His GA. By contrast, multipoint weak interactions involving a number of **chelate** moieties seem to be mainly responsible for adsorption of natural proteins. By using tailor-made affinity supports, a very simple procedure for one-step purification of GA with minimal adsorption of host proteins could be performed. Up to 20 mg of GA were adsorbed on each ml of **chelate** support while most of accompanying proteins were **hardly** adsorbed on such supports. Following few washing steps, the target enzyme was finally recovered (80% yield) by elution with 50 mM imidazole with a very high increment of specific activity (up to a 120 purification factor).

L12 ANSWER 11 OF 13 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 97095716 EMBASE

DOCUMENT NUMBER: 1997095716

TITLE: Folding-dependent binding of thyrotropin (TSH) and TSH receptor autoantibodies to the murine TSH receptor ectodomain.

AUTHOR: Vlas H.; Matsuoka N.; Graves P.N.; Magnusson R.P.; Davies T.F.

CORPORATE SOURCE: Dr. T.F. Davies, Mount Sinai Medical Center, Box 1055, 1 Gustave L. Levy Place, New York, NY 10029, United States

SOURCE: Endocrinology, (1997) 138/4 (1658-1666).

Refs: 38

ISSN: 0013-7227 CODEN: ENDOAO

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 003 Endocrinology

029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The mouse TSH receptor ectodomain (mTSHR-ecd) was amplified from murine thyroid complementary DNA and ligated into the pAcGP67B insect cell vector, and the nucleotide sequence was confirmed. Employing a baculovirus-insect cell system, the mTSHR-ecd (amino acids 22-415) was expressed as a **fusion** protein with the gp67 insect cell signal sequence at the NH<sub>2</sub>-terminus and a C-terminal six-histidine tag. Protein expression was assessed by Western blot using a murine monoclonal antibody (recognizing amino acids 22-35) and a rabbit antipeptide antibody (recognizing amino acids 397-415). These antibodies detected two principal species of mTSHR-ecd, one glycosylated (66 kDa) and one nonglycosylated (52 kDa), in cell lysates of infected insect cells. More than 10% of these species were present in a water-soluble (cytosolic) fraction. This fraction was then used to purify, under native conditions, 100-μg amounts of mTSHR-ecd using **nickel-nitrilo-triacetic (Ni-NTA)/resin chromatography**. The purified cytosolic mTSHR-ecd migrated as a homogeneous 66-kDa band visible on Coomassie blue-stained gels and was confirmed by Western blotting. We also purified the mTSHR-ecd from total cell lysates under denaturing conditions, followed by 'in vitro' refolding on the **Ni-NTA column**. Under these conditions, milligram amounts of soluble mT-SHR-ecd were obtained. This material consisted primarily of the 66-kDa glycosylated form, but in addition contained four or five lower molecular mass, partially glycosylated **intermediates** and the 52-kDa nonglycosylated form. Deglycosylation with either endoglycosidase F or H,

reduced all mTSHR-ecd glycosylated species to a 52-kDa non-glycosylated form. Both the cytosolic and refolded mTSHR-ecd preparations inhibited the binding of [125]TSH to the full-length human TSHR expressed in Chinese hamster ovary cells in a dose-dependent manner, with similar affinities. The affinity of such interactions was 3 orders of magnitude less than observed with native porcine TSHR and was further reduced by unfolding the mTSHR-ecd preparations. The cytosolic and refolded mTSHR-ecd were also recognized by hT-SHR autoantibodies in the serum of patients with hyperthyroid Graves' disease. Such autoantibody binding to mTSHR-ecd was also markedly reduced by unfolding the antigen. These results demonstrated the successful production of large quantities of well characterized, biologically active, mTSHR-ecd antigen. In addition, the data showed that although the ectodomain of the mTSHR bound TSH, intact holoreceptor may be required for high affinity ligand binding. Whether the transmembrane region is required for direct ligand binding, as seen for other G protein-linked receptors, or whether it is needed to stabilize the ligand binding to the ectodomain and maintain a correctly folded state, remains unclear.

L12 ANSWER 12 OF 13 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 97075938 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8918261  
 TITLE: A multifunctional vector system for heterologous expression of proteins in Escherichia coli. Expression of native and hexahistidyl **fusion proteins**, rapid **purification of the fusion proteins**, and removal of **fusion peptide** by Kex2 protease.  
 AUTHOR: Ghosh S; Lowenstein J M  
 CORPORATE SOURCE: Biochemistry Department, Brandeis University, Waltham, MA 02254, USA.  
 SOURCE: Gene, (1996 Oct 17) 176 (1-2) 249-55.  
 Journal code: 7706761. ISSN: 0378-1119.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199701  
 ENTRY DATE: Entered STN: 19970128  
 Last Updated on STN: 19980206  
 Entered Medline: 19970107

AB Vectors have been constructed for the general purpose of expressing foreign proteins in E. coli. These vectors allow the production in high yield of either native proteins or of **fusion proteins** which contain, at their amino terminus, the peptide Met Gly His6 Ser Gly Leu Phe Lys Arg/, where Leu Phe Lys Arg/ is the recognition site for Kex2 protease which cleaves at the site indicated by /. The His6 sequence is used as a ligand for the one-step affinity **purification** of the expressed **proteins on columns** containing Ni or Zn ions **chelated** to iminodiacetic acid-agarose. After affinity **chromatography**, the **purification peptide** is cleaved off with Kex2 protease from Saccharomyces cerevisiae. The vectors also allow site-directed mutagenesis and sequencing of the cloned gene to be expressed without any **intermediate** subcloning. For practical examples of over-expression, affinity purification, and removal of the **purification peptide**, we chose a high-molecular-weight protein, phospholipase C gamma 1 (PLC gamma 1, M(r) 148,000) and a low-molecular-weight protein, Hit-1 (M(r) 16,000). Both were obtained pure and in high yield. PLC gamma 1 was fully active; the function of



Hit-1 is not known. A set of companion vectors for co-expression of additional proteins has also been developed. These allow expression of proteins which enhance the production or activity of the protein of primary interest and of proteins which exhibit trans-interactions.

L12 ANSWER 13 OF 13 JICST-EPlus COPYRIGHT 2005 JST on STN

ACCESSION NUMBER: 960902029 JICST-EPlus  
TITLE: Preparation of the DHFR **Fusion** Protein of the Antigen Peptide for Human TIMP-1.  
AUTHOR: IMAMURA TAIZO; SHIBATA YASUKO; TAKIGUCHI HISASHI  
CORPORATE SOURCE: Nihon Univ., Sch. of Dent. at Matsudo  
SOURCE: Nichidai Koku Kagaku (Nihon University Journal of Oral Science), (1996) vol. 22, no. 3, pp. 222-232. Journal Code: Z0705A (Fig. 6, Ref. 23)  
ISSN: 0385-0145  
PUB. COUNTRY: Japan  
DOCUMENT TYPE: Journal; Article  
LANGUAGE: Japanese  
STATUS: New

AB The formation and degradation of extracellular matrix (ECM) components are closely related to the many physiological and pathological processes. The degradation of connective tissues is triggered by the activation of certain matrix metalloproteinase (MMP) during physiological and pathological turnover. This activation process is controlled by enzymatic cascade involved in the ECM metabolism. One additional characteristic of MMP family is that they are inhibited by specific proteinase inhibitors known as the tissue inhibitors of metalloproteinases (TIMPs). Both activated- and preactivated-MMPs are inhibited by TIMPs. These observations have suggested that MMPs activities are tightly regulated by both positive and negative control systems in several tissues. In this study, we have attempted to construct the recombinant **fusion** protein consisting of dihydrofolate reductase (DHFR) and TIMP-1 (rDHFR-TIMP-1 protein) to prepare rabbit IgG against human TIMP-1. To obtain the **fusion** protein, the plasmid pQE40 (the QIA expressionist kit) was used. This plasmid is composed of several gene cassettes including a promoter, 6 His, DHFR, and MCS. To obtain the cDNA for TIMP-1, total RNAs from fibroblastic cells of human periodontal ligament were used as a template for RT-PCR. PCR amplified TIMP-1 gene was gel purified, and inserted into the pGEM-3Zf(+) and pQE40. After the confirmation of TIMP-1 cDNA insert by DNA sequencing, DNA fragment spanning from promoter to cDNA insert was further subcloned into the pHSG298 to obtain vast amount of the **fusion** protein following the induction with IPTG. rDHFR-TIMP-1 **fusion** protein was present in the insoluble fraction of transformant. Because the NH2-terminal end of this recombinant **fusion** protein is the 6 His tag amino acid stretch, **purification** of this **protein** was facilitated by passing through onto the Ni-NTA resin column. (author abst.)

=> d his ful

FILE 'REGISTRY' ENTERED AT 14:53:15 ON 04 FEB 2005

L1 7 SEA ABB=ON (IRON OR CALCIUM OR ALUMINUM OR NICKEL OR ZINC OR  
COBALT OR COPPER)/CN

FILE 'HCAPLUS' ENTERED AT 14:53:50 ON 04 FEB 2005

L2 111827 SEA ABB=ON (?PEPTID? OR ?PROTEIN?) (4A) (?PURIF? OR ?WASH? OR  
?EXTRACT? OR ?ELUT?)  
L3 3656 SEA ABB=ON L2 AND (?CHELAT? OR ?RESIN?)  
L4 1288 SEA ABB=ON L3 AND (L1 OR ?IRON? OR FE OR ?CALCIUM? OR CA OR  
?ALUMINUM? OR AL OR ?NICKEL? OR NI OR ?ZINC? OR ZN OR ?COBALT?  
OR CO OR ?COPPER? OR CU)  
L5 229 SEA ABB=ON L4 AND (?FUSE? OR ?FUSION?)  
L6 177 SEA ABB=ON L5 AND (?CHROMATOG? OR ?BUFFER? OR ?COLUMN?)  
L7 4 SEA ABB=ON L6 AND (?HARD? OR ?INTERMED?)  
L8 6 SEA ABB=ON L6 AND KIT?  
L9 9 SEA ABB=ON L7 OR L8  
L10 9 SEA ABB=ON L7 OR L8 OR L9

*9 cit's from CA Plus*

FILE 'MEDLINE, BIOSIS, EMBASE, JAPPIO, JICST-EPLUS, COMPENDEX' ENTERED AT  
14:57:27 ON 04 FEB 2005

L11 15 SEA ABB=ON L10  
L12 13 DUP REMOV L11 (2 DUPLICATES REMOVED)

*13 cit's from other  
databases*

=&gt; d ibib abs ind l3 1-3

L3 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2004:759732 HCAPLUS  
DOCUMENT NUMBER: 141:273989  
TITLE: Purification of fusion proteins using immobilized  
bi-metal affinity chromatography  
INVENTOR(S): Tchaga, Grigoriy S.; Jokhadze, George  
G.  
PATENT ASSIGNEE(S): USA  
SOURCE: U.S. Pat. Appl. Publ., 20 pp., Cont.-in-part of U.S.  
Pat. Appl. 2002 164,718.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004180415	A1	20040916	US 2004-762588	20040121
US 2002164718	A1	20021107	US 2001-858332	20010515
PRIORITY APPLN. INFO.:			US 2001-858332	A2 20010515
			US 2003-441804P	P 20030121
			US 1998-101867P	P 19980925
			US 1999-404017	B2 19990923

AB The present invention relates to IMAC (Immobilized Metal Affinity Chromatog.). The present invention provides methods of purifying proteins that include a metal ion affinity peptide. The methods generally involve contacting a fusion protein that includes a metal ion affinity peptide with at least two different metal ion chelating resins. In certain representative embodiments, the methods include contacting a fusion protein with a first metal ion chelate resin having a first immobilized metal ion; eluting any bound protein from the first metal ion chelate resin, to produce an eluate; contacting the eluate with a second metal ion chelate resin having a second immobilized metal ion; and eluting any bound protein from the second metal ion chelate resin. Also provided are kits for use in practicing the subject methods. An illustrative purification protocol for Bi-MAC (Bi-Metal Affinity Chromatog.) is shown. The subject methods find use in a variety of protein purification applications.

ICM C12N009-00

NCL 435183000; 530412000

CC 9-3 (Biochemical Methods)

ST fusion protein purifn bimetal affinity chromatog IMAC; metal ion chelating resin affinity peptide fusion protein purifn

IT Affinity chromatography

(IMAC; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Washing

(buffer for; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Extractants

(buffer; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Denaturants

(denaturing conditions; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Buffers

(extraction, wash and elution; purification of fusion proteins using immobilized

bi-metal affinity chromatog.)

IT Cations  
(hard and intermediate metal ions; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Affinity chromatographic stationary phases  
(metal ion affinity peptide; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Peptides, uses  
RL: NUU (Other use, unclassified); USES (Uses)  
(metal ion affinity peptide; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Immobilization, molecular or cellular  
(of metal ions; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Liquid chromatographic columns  
Test kits  
(purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT Fusion proteins (chimeric proteins)  
RL: PUR (Purification or recovery); PREP (Preparation)  
(purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT 7429-90-5, Aluminum, uses 7439-89-6, Iron, uses 7440-70-2, Calcium, uses 14127-61-8, Calcium 2+, uses 20074-52-6, Iron 3+, uses 22537-23-1, Aluminum 3+, uses  
RL: NUU (Other use, unclassified); USES (Uses)  
(immobilized, hard metal ion; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT 7440-02-0, Nickel, uses 7440-48-4, Cobalt, uses 7440-50-8, Copper, uses 7440-66-6, Zinc, uses 14701-22-5, Nickel 2+, uses 15158-11-9, Copper 2+, uses 22541-53-3, Cobalt 2+, uses 23713-49-7, Zinc 2+, uses  
RL: NUU (Other use, unclassified); USES (Uses)  
(immobilized, intermediate metal ion; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

IT 54017-28-6 85139-13-5 91859-00-6 92000-72-1 98849-88-8  
158734-08-8 444311-97-1 474426-21-6 474426-23-8 474426-25-0  
474426-27-2 474426-29-4  
RL: PRP (Properties)  
(unclaimed sequence; purification of fusion proteins using immobilized bi-metal affinity chromatog.)

L3 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:850241 HCAPLUS

DOCUMENT NUMBER: 137:365958

TITLE: Metal ion affinity peptides and methods for using the same in protein purification methods

INVENTOR(S): Tchaga, Grigoriy S.; Jokhadze, George G.

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 23 pp., Cont.-in-part of U. S. Ser. No. 404,017, abandoned.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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US 2002164718	A1	20021107	US 2001-858332	20010515

US 2004180415	A1	20040916	US 2004-762588	20040121
PRIORITY APPLN. INFO.:			US 1998-101867P	P 19980925
			US 1999-404017	B2 19990923
			US 2001-858332	A2 20010515
			US 2003-441804P	P 20030121

OTHER SOURCE(S): MARPAT 137:365958

AB The present invention provides metal ion affinity peptides, fusion proteins comprising metal ion affinity peptides, and polynucleotides encoding the fusion proteins. The invention further provides recombinant vectors comprising subject polynucleotides, and host cells comprising the recombinant vectors. The invention further provides methods and kits for purifying a fusion protein comprising a metal ion affinity peptide.

IC ICM C12P021-02  
ICS C12N005-06; C12P021-04; C07K014-435; C07K007-08

NCL 435069700

CC 9-3 (Biochemical Methods)  
Section cross-reference(s): 3, 6

ST metal ion affinity chromatog genetic recombination fusion protein purifn

IT Peptides, uses  
RL: ARG (Analytical reagent use); PRP (Properties); ANST (Analytical study); USES (Uses)  
(affinity; metal ion affinity peptides and methods for using the same in protein purification methods)

IT Chromatographs  
(columns, purification kit; metal ion affinity peptides and methods for using the same in protein purification methods)

IT Buffers  
(extraction, washing, elution; metal ion affinity peptides and methods for using the same in protein purification methods)

IT Prokaryota  
(host cell; metal ion affinity peptides and methods for using the same in protein purification methods)

IT Affinity chromatography  
Genetic vectors  
Immobilization, molecular or cellular  
Purification  
Recombination, genetic  
Washing  
(metal ion affinity peptides and methods for using the same in protein purification methods)

IT Metals, uses  
RL: ARG (Analytical reagent use); PRP (Properties); ANST (Analytical study); USES (Uses)  
(metal ion affinity peptides and methods for using the same in protein purification methods)

IT Proteins  
RL: PRP (Properties); PUR (Purification or recovery); PREP (Preparation)  
(metal ion affinity peptides and methods for using the same in protein purification methods)

IT Fusion proteins (chimeric proteins)  
RL: ARG (Analytical reagent use); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); USES (Uses)  
(peptide fused to metal ion affinity peptide; metal ion affinity peptides and methods for using the same in protein purification methods)

IT Polynucleotides  
RL: ARG (Analytical reagent use); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); USES (Uses)  
(recombinant; metal ion affinity peptides and methods for using the same in protein purification methods)

IT 474426-21-6 474426-23-8 474426-25-0 474426-27-2 474426-29-4

RL: ARG (Analytical reagent use); PRP (Properties); ANST (Analytical study); USES (Uses)

(amino acid sequence; metal ion affinity peptides and methods for using the same in protein purification methods)

IT 14127-61-8, Ca<sup>2+</sup>, uses 14701-22-5, Ni<sup>2+</sup>, uses 15158-11-9, Cu<sup>2+</sup>, uses  
20074-52-6, Fe<sup>3+</sup>, uses 22537-23-1, Al<sup>3+</sup>, uses 22541-53-3, Co<sup>2+</sup>, uses  
23713-49-7, Zn<sup>2+</sup>, uses

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(metal ion affinity peptides and methods for using the same in protein purification methods)

IT 475131-38-5 475131-39-6 475131-40-9 475131-41-0 475131-42-1  
475131-43-2

RL: PRP (Properties)  
(unclaimed nucleotide sequence; metal ion affinity peptides and methods for using the same in protein purification methods)

IT 92000-72-1 98849-88-8 444311-97-1 475084-76-5 475084-78-7  
475131-35-2 475131-37-4

RL: PRP (Properties)  
(unclaimed sequence; metal ion affinity peptides and methods for using the same in protein purification methods)

L3 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:736412 HCAPLUS

DOCUMENT NUMBER: 131:348793

TITLE: Compositions and methods for protein purification based on a metal ion affinity site

INVENTOR(S): Tchaga, Grigoriy; Jokhadze, George G.

PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA

SOURCE: PCT Int. Appl., 42 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9957992	A1	19991118	WO 1999-US10662	19990514
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9941877	A1	19991129	AU 1999-41877	19990514
PRIORITY APPLN. INFO.:			US 1998-78687	A 19980514
			WO 1999-US10662	W 19990514

AB The present invention describes introducing metal ion affinity sequences into recombinant proteins to allow for purification and/or immobilization of these proteins. The present invention provides a fusion protein, comprising: a protein of interest fused at its amino-terminus or carboxy-terminus to at least one affinity peptide, said fusion protein having a formula R1-(HX<sub>n</sub>)<sub>m</sub>-R2, wherein R1 or R2 is said protein of interest, n = 1-8, m = 2-30, and wherein if n = 1 for more than two adjacent units of HX, at least one X must be asparagine, phenylalanine, tryptophan, tyrosine, lysine, methionine, arginine, glutamine, or cysteine.

IC ICM A23J001-00

ICS C12N015-63; C12N015-00; C12N001-21; C12P021-04; A61K038-00;  
C07K001-00; C07H021-04

CC 9-16 (Biochemical Methods)

ST compn protein purifn metal ion affinity

- IT Peptides, biological studies  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(Affinity; compns. and methods for protein purification based on a metal ion affinity site)
- IT Affinity  
Bond cleavage  
Cations  
Chelating agents  
Composition  
DNA sequences  
Extraction  
Immobilization, biochemical  
Muscle  
Purification  
(compns. and methods for protein purification based on a metal ion affinity site)
- IT Fusion proteins (chimeric proteins)  
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
(compns. and methods for protein purification based on a metal ion affinity site)
- IT Proteins, general, preparation  
RL: BPN (Biosynthetic preparation); PEP (Physical, engineering or chemical process); PUR (Purification or recovery); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)  
(compns. and methods for protein purification based on a metal ion affinity site)
- IT Amino acids, biological studies  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(compns. and methods for protein purification based on a metal ion affinity site)
- IT Proteins, specific or class  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(green fluorescent, UV mutant; compns. and methods for protein purification based on a metal ion affinity site)
- IT Genetic vectors  
(recombinant; compns. and methods for protein purification based on a metal ion affinity site)
- IT 52-90-4, Cysteine, biological studies 56-85-9, Glutamine, biological studies 56-87-1, Lysine, biological studies 60-18-4, Tyrosine, biological studies 63-68-3, Methionine, biological studies 63-91-2, Phenylalanine, biological studies 70-47-3, Asparagine, biological studies 71-00-1, Histidine, biological studies 73-22-3, Tryptophan, biological studies 74-79-3, L-Arginine, biological studies  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(compns. and methods for protein purification based on a metal ion affinity site)
- IT 250266-36-5P  
RL: BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PUR (Purification or recovery); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)  
(compns. and methods for protein purification based on a metal ion affinity site)
- IT 9001-92-7, Protease  
RL: CAT (Catalyst use); USES (Uses)  
(compns. and methods for protein purification based on a metal ion affinity site)
- IT 14701-22-5, uses  
RL: NUU (Other use, unclassified); PEP (Physical, engineering or chemical

process); PROC (Process); USES (Uses)  
(compns. and methods for protein purification based on a metal ion affinity site)

IT 9001-60-9P, Lactate dehydrogenase  
RL: PUR (Purification or recovery); PREP (Preparation)  
(compns. and methods for protein purification based on a metal ion affinity site)

IT 250673-92-8P  
RL: BPN (Biosynthetic preparation); PEP (Physical, engineering or chemical process); PUR (Purification or recovery); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)  
(nucleotide sequence; compns. and methods for protein purification based on a metal ion affinity site)

IT 250676-83-6, 3: PN: WO9957992 SEQID: 3 unclaimed DNA 250676-84-7, 4: PN: WO9957992 SEQID: 4 unclaimed DNA  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; compns. and methods for protein purification based on a metal ion affinity site)

IT 250662-75-0 250676-82-5  
RL: PRP (Properties)  
(unclaimed protein sequence; compns. and methods for protein purification based on a metal ion affinity site)

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT